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(57) Abstract

Recombinant DNA molecules comprising a nucleotide sequence which codes for the atopy related antigens Ka, Kb, ara-3 or ara-4 (as defined in figures 1 - 4) or homologous forms thereof or at least one epitope thereof, or a nucleotide sequence which hybridizes with such a sequence under conditions of high stringency. Recombinant DNA expression vectors and host cells comprising these DNA molecules. Polypeptides encoded by the recombinant DNA molecules. Diagnostic and therapeutic methods employing an atopy related antigen, in particular the Ka, Kb, ara-3, ara-4 and ara-5 antigens that are based on the sequences defined in figures 1 - 5.

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RECOMBINANT MOLECULES, PROTENS/POLYPEPTIDES, HOST SYSTEMS AND DIAGNOSTIC AND THERAPEUTIC METHODS FOR ATOPY.

INTRODUCTION

Atopic dermatitis represents an itchy cutaneous disease which is mostly associated with a high level of total serum IgE and a pronounced TH-2 cellular immune response (1-5). It has been shown that in early atopic dermatitis lesions vascular hypertrophy, endothelial cell activation and skin infiltration with mast cells, basophils, eosinophils and T-cells occurs (6). In addition Langerhans cells and monocytes of atopic patients were found to express increased amounts of the high affinity receptor for IgE (7-8). T-cells obtained from atopic dermatitis patients mostly belong to the TH-2 subset secreting more IL-4 whereas interferon-γ levels were found to be reduced in atopic dermatitis (9-14). It can thus be assumed that atopic dermatitis such as Type I allergy belongs to diseases with a TH-2 activation.

In Type I allergy the environmental allergens (pollen proteins, mite proteins...) can be clearly defined (15,16) and the specificity of IgE-antibodies against environmental allergens significantly correlates with the course of the disease. In the case of atopic dermatitis the high levels of serum IgE do not correlate with a certain sensitization pattern and no specific antigen spectrum could be defined as yet. Although it has been shown that atopic dermatitis patients display IgE-reactivity to various mite, pollen and food allergens (17) no clear association between a certain allergen source and the disease could be established.

Based on the notion of a similarity between a birch pollen allergen identified as the cytoskeletal protein, profilin and human profilin (2) we investigated whether certain atopic diseases might be associated with autoreactivity against human proteins. Despite the ability of plant and mammalian profilins to interact equally well with plant and mammalian actins thus suggesting a high degree of structural similarity of different profilins (27,20) no direct evidence could be provided that IgE-

autoreactivity against profilins can trigger severe symptoms of atopy. Suggestions about the involvement of profilin as an autoantigen in allergic diseases and atopy have also been put forward in the patent litterature (35). Neither in this latter publication any real substantiation of the idea was presented.

During the priority year the results presented herein was partially published for the Ka antigen (12th European Immunology Meeting, June 14-17, Barcelona, European Federation of Immunological Science).

Definitions

If not otherwise specified, the expressions "Ka antigen" and "Kb antigen" mean any peptide/protein in which the amino acid sequences (minus underlined parts) given in figure 1 and 2, respectively, or parts thereof unique for Ka and Kb can be retrieved. For example the complete native forms and genetic engineered variants containing different combinations of one or more unique parts/epitopes of the complete native forms.

By the expression "a polypeptide displaying the antigenicity of the Ka or Kb antigen" is meant any peptide displaying antigenic crossreactivity with one or more epitopes that are unique for the Ka or Kb antigen. One can check for crossreactivity by inhibition experiments.

Preparations of the Ka or Kb antigen are normally substantially pure in the sense that their content of other proteins/peptides originating from the source in which they have been produced normally is < 50 % such as < 25 % or < 10 % or < 1% (w/w).

During the priority year the IgE reactive autoantigens of the type concerned herein have been named atopy related antigens (ara). Ara-1 is the Kb and ara-2 the Ka antigen. The definitions/statements concerning Ka and Kb are valid also for the atopy related antigens (ara-3, ara-4 and ara-5), the cDNA of which are partially presented in figures 3-5. The term autoantigen below refer to atopy related antigens, if not otherwise specified.

By hybridization during high stringency is meant conditions giving essentially the same or better specificity as the conditions applied in the experimental part for estimating the sizes of the native forms of the Ka and Kb antigens via hybridization to the corresponding mRNAs.

The term epitope (B-cell epitope) in the context of the present invention identifies a region on an antigen where antibodies can bind, for instance IgE or IgG antibodies that may be of mammalian, particularly human, origin. The term primarily designates the IgE-binding site for exclusively one antibody with a given specificity.

Objectives

The objectives of the invention are to provide simple, better and more reliable in vitro an in vivo tests for atopy, in particular atopic dermatitis, as well as therapeutic methods for this disease.

The invention

One aspect of the invention is a recombinant DNA molecule comprising

i) a nucleotide sequence which codes
   - i:1) for a polypeptide displaying the antigenicity of the atopy related antigens Ka, Kb, ara-3 or ara-4 or
   - 1:2) for a polypeptide that is homologous to > 50 %, such as > 60 %, > 70 % or > 80 % to these atopy related antigens, or
   - i:3) for a peptide comprising at least one epitope unique for (a) these atopy related antigens or (b) a peptide that is homologous to > 50 %, such as > 60 %, > 70 % or > 80 % to the Ka or Kb antigen, or

ii) a nucleotide sequence which hybridizes with such a sequence under conditions of high stringency.

A second aspect of the invention is a recombinant DNA expression vector or cloning system comprising an expression

control sequence operatively linked to a nucleotide sequence defined in (i) and (ii) above.

A third aspect of the invention is a host cell or host system containing a recombinant DNA molecule or a recombinant expression vector as defined above.

A fourth aspect of the invention is a polypeptide comprising the amino acid sequence of one of the atopy related antigens defined above or at least one epitope unique for these atopy related antigens. Preferred modes of this aspect comprise that the polypeptide have been produced recombinantly or synthetically. Another preferred mode comprises that the polypeptide has been derivatized in the sense that it is a) linked to a water-insoluble phase by physical adsorption or by a covalent bond, b) conjugated covalently to an analytically detectable group (label), and/or c) covalently linked to an additionally polypeptide, for instance by being recombinantly produced in the form of a fusion protein comprising the additional protein.

The water-insoluble phase may be a polymer that is water-insoluble and selected from insoluble forms of polysaccharides and their derivatives, for instance dextran, pullulan, agarose, cellulose etc, or synthetic polymers, preferably vinyl polymers, such as polyacrylamides, polyacrylates, polystyrene, polyvinyl alcohol, polyvinyl ethers etc. The physical form of the insoluble phase may be: walls of microtitre wells, spheres, rods, sheets, strips, pads etc, said physical forms may be porous or non-porous.

The additional protein may be β-galactosidase, GST or lambda cII protein or any other polypeptide which can be expressed in a prokaryotic or eukaryotic cell.

A fifth aspect of the invention is a method for in vitro diagnosis of atopy or inflammation in a mammalian individual, such as a human individual, by detecting/determining abnormal levels (preferably elevated) of antibodies directed against an atopy related antigen. This method comprises detection of the reaction of IgE in a body fluid sample from the individual with

an IgE autoantigen from the same species as the individual or an IgE hapten/autoantigen crossreacting with the IgE autoantigen, optionally derived from another source. The formation of an IgE immune complex is taken as an indication that the individual is suffering from atopy or inflammation.

The atopy preferred to diagnose at the filing date of this specification is atopic dermatitis and intrinsic asthma bronchiale with elevated IgE.

The preferred autoantigens to be used in this aspect of the invention are the atopy related autoantigens as defined above and autoantigens comprising an epitope of ara-5, the cDNA sequence of which is given in figure 5.

The body fluid sample contains IgE is mostly derived from blood, such as a whole blood sample, a serum sample or a plasma sample. Also other fluids such as CSF, urine etc may have a potential use.

One preferred protocol of the method is to bring the autoantigen in insoluble form or in insolubilizable form into contact with the sample under conditions permitting formation of an immune complex between the autoantigen and antibodies present in the sample, whereafter the amount of complexed IgE antibodies is detected, where appropriate after insolubilization of the formed IgE-autoantigen complex. Detection may preferably be done by the use of labeled ani-IgE antibody.

Another preferred protocol of the method employs labeled autoantigen, and the immune complex between the autoantigen and sample IgE is formed in a soluble form that in the course of being quantitated is insolubilized by being contacted with water-insoluble or water-insolubilizable anti-IgE antibodies, preferably linked to the solid phases given above.

The above-mentioned methods including the preferred protocols may analogously be used for the determination of antibodies of other classes, e.g. IgG.

A sixth aspect of the invention is a method to measure in vitro a cellular reaction against an atopy related autoantigen comprising the step of stimulating or inhibiting the cellular

reaction with the atopy related autoantigen. Examples of suitable autoantigens contain at least one atopy related epitope present in either Ka, Kb, ara-3, ara-4 or ara-5. The stimulation/inhibition may be performed by measuring histamine liberated from basophils or mast cells which have been loaded with IgE specific for an atopy related autoantigen plus the autoantigen or with IgE complexed to the atopy related autoantigen. Measuring can also be done through proliferation of autoantigen specific T cells (for example as ^{3}H thymidine uptake). This method too may be used for the diagnosis of atopy as defined for the fifth aspect of the invention.

A seventh aspect of the invention is a method of treatment of an mammalian individual, in particular a human being, suffering from atopy wherein an effective amount of a polypeptide comprising an epitope of an atopy related autoantigen as defined above is administered to said individual. The same protocols/modes as used with pollen extracts are potentially applicable. In addition one might think about clearing a patient's plasma by affinity adsorption to an insolubilized atopy related autoantigen. All modes of therapy which are currently considered for humoral autoimmune diseases (tolerance induction etc) may also be applied to those patients who display IgE-autoreactivity.

An eighth aspect of the invention is a method to diagnose inflammation or atopy by detecting/determining the prescence of abnormal levels (normally elevated) of an IgE- or IgG-atopy related antigen as defined above (preferably related to the Ka, Kb, ara-3, ara-4 or ara-5 antigens). The protocols to be employed comprise adsorbing out the atopy related antigen from a body sample by use of an antibody and detecting in a known per se mannner the antigen. The antibody may be directed against the atopy related antigen or against an antibody complexed to the atopy related antigen, for instance an anti-IgE antibody in case the antigen is complexed to IgE in the sample.

At the filing of this specification, the preferred modes of all eighth aspects made use of recombinant DNA molecules (aspects 1-3) or where appropriate polypeptides (aspects 4-7) having the sequences set out in figures 1-4 or degenerative variants thereof, or one or more Ka, Kb, ara-3 or ara-4 unique portion of said sequences or variants. For aspects 5-7, the preferred modes at the same time also comprised using ara-5 (see figure 5) and the analogous variants of this autoantigen.

As determined by Northern blot the atopy related autoantigens Ka, Kb, ara-3, ara-4 and ara-5 appear to be expressed in histogenetically unrelated cells. We have also determined that they are expressed on keratinocytes and endothelial cells.

The invention is defined in the appending claims.

EXPERIMENTAL PART

MATERIALS AND METHODS

IgE-immunoscreening of a human keratinocyte expression cDNA library

In a study that was unpublished when this specification was filed, we have shown that atopic dermatitis patients display IgE-autoreactivity against nitrocellulose blotted human proteins of different cell types including keratinocytes, endothelial cells, fibroblasts, platelets and mononuclear cells. The phenomenon of IgE-autoreactivity with human proteins was pronounced in atopic dermatitis patients suggesting that IgE-autoimmune mechanisms might contribute to the pathogenesis of the disease. To further characterize the IgE-autoantigens, serum IgE from an atopic dermatitis patient was used to screen a randomly primed expression cDNA library prepared from the human keratinocyte cell line (A431).

A lambda gt 11 expression cDNA library (Clontech, Palo Alto, USA) was screened with serum IgE from a patient suffering from atopic dermatitis. In brief, 500 000 phage particles of the keratinocyte expression cDNA library were plated at a density of 20 000 particles per plate (140 mm diameter) by infecting E. coli Y1090 at 43°C (21). After plaques were visible the plates were

overlaid with nitrocellulose filters (Schleicher & Schuell, Dassel, Germany), soaked in 10 mM IPTG for the induction of recombinant protein synthesis. Lambda gt 11 phage without insert were plated as well and overlaid with nitrocellulose filters for preadsorption of the patient serum before immunoscreening to reduce background reactivity of IgE with E. coli phage proteins. Serum IgE from a patient suffering from atopic dermatitis was diluted 1:10 and preincubated with nitrocellulose filters containing E. coli lambda gt/proteins that had been incubated twice for 5 minutes and once for 30 minutes in buffer A (50 mM sodium phosphate pH 7.5, 0.5 % BSA, 0.5 % Tween 20, 0.05 % NaN_3) for 1 hour at 4°C. The serum was further diluted in buffer A to a final dilution of 1:20 and incubated overnight at 4°C with nitrocellulose filters containing plaquelifts of the recombinant phage. Filters were then washed as described for the blocking and incubated with 1:10 diluted ^{125}I-labeled anti-human IgE (Pharmacia Diagnostics, Uppsala, Sweden) overnight at room temperature. The filters were then washed again as described for the blocking and exposed to Kodak X-OMAT S Films at -70°C using intensifying screens (Kodak, Heidelberg, Germany). Preincubation of the atopic dermatitis patient serum with E. coli/phage proteins was found to be critical for a reduction of the background binding of IgE to facilitate the discrimination of positive clones. Recombinant phage particles were enriched by IgE-screening to homogeneity by two rounds of recloning before preparation of phage DNA.

Characterization of cDNA clones coding for human IgE-autoantigens

More than 30 IgE-binding phage clones were obtained by the IgE-immunoscreening with serum IgE from an atopic dermatitis patient. Phage DNA was isolated using a plate lysate method and according to a restriction analysis of two clones which contained small cDNA inserts (approximately 300 base pairs) thus representing small proteins or IgE-epitopes were subcloned into plasmid pUC18. In brief, phage DNA was digested with Kpn I and Sac I to excise the cDNA inserts flanked by approximately 1000 base pairs of

lambda gt 11 sequence thus allowing he cDNA to be subcloned in known orientation into plasmid pUC18. Using lambda gt 11 forward and reversed primer (Clontech, Palo Alto, USA), a sequencing kit (Pharmacia Biotech AB, Uppsala, Sweden) and ^{35}S dCTP (NEN, Stevenhage, U.K.), the DNA sequence of both strands could be obtained (22) and allowed the determination of the orientation and correct reading frame within the β-galactosidase fusion portion. The cDNA and deduced amino acid sequence of clone Ka was compared with EMBL/SwissProt library and GenBank.

For determination of the full transcript size of the Ka and Kb mRNA the corresponding cDNA insert was isolated by Eco R I digest of the plasmid subclone. The agarose gel purified cDNA fragment was radiolabeled using ^{32}P dCTP according to Feinberg and Vogelstein (23) and was hybridized with nitrocellulose blotted RNA (approximately 15 µg) prepared from the human keratinocyte cell line (A431)(24) or from a human mast cell line (HMC-1) by denaturing agarose gel electrophoresis.

Expression and purification of recombinant IgE-autoantigens

The Kb IgE-autoantigen was expressed as β-galactosidase fusion protein upon infection of E. coli Y1089 with recombinant phage. Lambda gt 11 phage (negative control) without inserts were used likewise to induce synthesis of β-galactosidase which served as a control protein. β-galactosidase and the recombinant IgE autoantigen fragments were purified using a β-galactosidase affinity matrix (Lambda ProtoSorb, Promega, Maddison, USA).

Binding of IgE antibody of atopic patients to recombinant IgE autoantigens

Purified β-galactosidase, Kb fusion protein was separated by SDS-Page using a 8 % gel (25) and blotted to nitrocellulose (Schleicher & Schuell, Dassel, Germany) (26). Strips containing the purified proteins were blocked as described for the plaquelifts in buffer A and incubated with diluted sera from atopic dermatitis patients, pollen allergic patients, non-allergic individuals or buffer without addition of serum. IgE-

binding sera were diluted 1:10. Incubation was done as described for the plaquelifts at 4°C over night. Bound serum IgE was detected with ^{125}I labeled anti-human IgE antibodies (Pharmacia Diagnostics, Uppsala, Sweden).

Enhanced expression of Kb upon heat shock of cultured keratinocytes.

The human keratinocyte cell line (A431) was incubated under lack of oxygen at 37°C and 43°C for different periods of time. Proteins were separated by SDS-PAGE and blotted to nitrocellulose. Nitrocellulose strips were incubated with sera reacting with Kb at 55-60 kD, an atopic patient without specificity for Kb and control sera. A buffer control without addition of serum was also included.

RESULTS AND DISCUSSION

The IgE-immune screening procedure which was used for the isolation of cDNAs coding for IgE-autoantigens was the same which had been used in earlier studies for the successful characterization of cDNAs coding for exogenous allergens (pollen (18,27,28,29) and dog allergens (30). To reduce non-specific IgE-reactivity of the serum from atopic patients during immunoscreening the serum was preadsorbed with E. coli/phage proteins to minimize background reactivity. Despite the elevated levels of total IgE and the remarkable IgE-reactivity of atopic dermatitis patients with E. coli proteins it was thus possible to isolate and enrich phage clones coding for human IgE-autoantigens. The cDNA inserts of two IgE-binding clones designated Ka and Kb were excised with Kpn I and Sac I from the phage DNA together with the flanking lambda gt 11 DNA and subcloned into plasmid pUC 18. This allowed the determination of the correct reading orientation and reading frame of the cDNA within the β-galactosidase fusion protein portion. Figure 1 shows the complete cDNA and deduced amino acid sequence of the Ka cDNA and the cDNA sequence of Kb is displayed in Figure 2. The cDNA of

Ka showed significant sequence identities with human cDNAs which had been isolated from skeletal muscle and hepatocyte cDNA libraries in the course of human cDNA sequencing projects (31). The identities were found by alignment of the Ka cDNA sequence with

- a partial human cDNA (GenBank accession number: Z28824; clone HSBA0F011) coding for a transcribed sequence isolated from a human skeletal muscle cDNA library during the Genexpress cDNA program, and
- a cDNA clone which was isolated from a human hepatocyte cDNA library (GenBank accession number: D12194; clone: HUM000S318; Figure 3 B)).

Both homologous cDNAs code represent incomplete fragments and no biological function of the corresponding proteins could be established as yet. It might however be assumed that the Ka cDNA and the identical homologous cDNAs code for a protein which is expressed in different cell types (muscle cells, hepatocytes, keratinocytes) and therefore might represent a rather ubiquitous and conserved protein. This corroborates our previous Western blot results showing that atopic patients display IgE reactivity to proteins of similar molecular weight present in different cell types.

To estimate the size of the complete Ka and Kb transcripts, Northern blot hybridizations were performed. The ^{32}P labeled Ka cDNA hybridized with RNA from human keratinocytes at approximately 1800 nucleotides indicating that a corresponding protein of approximately 30-40 kD might be expected. Since the Ka cDNA coded only for an open reading frame of 93 amino acid residues, the polypeptide represents an IgE-epitope of the complete IgE-autoantigen against which a rather low percentage of atopic patients displayed IgE-reactivity.

The ^{32}P labeled Kb cDNA hybridized between the 26S and 18S RNA indicating a transcript size of approximately 2500-3000 nucleotides and a corresponding protein of approximately 50-60 kD. The cDNA coding for Kb was isolated from a keratinocyte cDNA library (A431) but apparently can be found in different cell

types. By Western blotting using a rabbit anti-recombinant Kb antiserum, Kb could be detected in keratinocytes, endothelial cells and fibroblasts.

Immunoblotting of the purified Kb-β-galactosidase fusion and β-galactosidase alone after separation by 12 % SDS-PAGE showed that the Kb-fusion but not β-galactosidase could be successfully used to block IgE-binding against a protein between 50-60 kD in human keratinocytes. It was further shown that atopic dermatitis patients displayed IgE reactivity with the recombinant Kb IgE-autoantigen but not to β-galactosidase which was used as a control protein. Non-allergic individuals did not show IgE-reactivity with the recombinant IgE-epitope nor with β-galactosidase. In addition to the IgE-reactivity, binding of patients IgG to the Kb fusion protein was observed (data not shown), indicating that the interaction between Kb and human IgE and IgG antibodies reflects the interaction of the Fab part of the antibodies with the epitope. This is of particular importance in view of earlier reports regarding IgE-dependent histamine releasing factors which were assumed to interact with the constant parts of human IgE (32,33). The occurrence of IgE-dependent histamine releasing factors has been described to be frequently associated with severe forms of atopy and atopic dermatitis, and it was concluded that these factors might be responsible for the increased capacity of basophils and mast cells of patients to release histamine without antigen stimulation.

Our results might be interpreted that IgE-dependent histamine releasing factors could represent IgE-autoantigens which are complexed with serum IgE. Circulating IgE-autoantigen complexes might then be able to activate mast cells and basophils. Such circulating immune complexes (IgG) were already demonstrated in atopic dermatitis patients (34) and it is likely that also IgE immune complexes might occur in atopic dermatitis patients sera. Similarly as was noted for IgE-dependent histamine releasing factors we have observed that basophils from atopic patients with IgE-reactivity to human proteins showed an increased ability to

spontaneously release histamine. In a final experiment it is demonstrated that the expression of Kb protein is significantly increased in stressed cells indicating that Kb might belong to a family of stress proteins.

Our results clearly prove that atopic dermatitis patients display IgE-autoreactivity to recombinant human IgE-epitopes and the working hypothesis is coined that atopic dermatitis represents an IgE-autoimmune disease. The described recombinant IgE-autoantigens may be extremely useful for diagnosis of severe atopy and Kb might be considered as a general inflammation marker. In addition to the usefulness for diagnostic procedures the described IgE-autoantigens might be used for attempts to induce immunological tolerance in atopic patients.

By applying the same methodology as described above we have, during the priority year, been able to discover three more IgE binding autoantigens (ara-3, ara-4 and ara-5) that are involved in atopy as described above. See under the legends to figure 3-5.

CHARACTERISTICA OF THE DETERMINED SEQUENCES

Sequence No 1. The cDNA sequence and deduced amino acid sequence of the Ka IgE-epitope fused to β-galactosidase.

The in situ sequence of the Ka cDNA fused to β-galactosidase was determined using lambda gt 11 forward and reversed sequencing primers. The Eco R I restriction sites are printed in italics and the sequence portion coding for β-galactosidase is underlined. A 93 amino acids long open reading frame is encoded by the Ka cDNA which is terminated by a stop codon TGA indicated by an asterisk.

Sequence No 2. cDNA and deduced amino acid sequence of IgE-autoantigen Kb fused to β-galactosidase.

The cDNA sequence of Kb fused to the sequence coding for β-galactosidase (underlined) is displayed. The deduced amino acid sequence is displayed below the nucleotide sequence. The Eco R I restriction sites are printed in italics. Due to an Eco R I linker dimer the open reading frame comprising 1347 nucleotides

is fused in frame to β-galactosidase. Both strands of the cDNA sequence were determined according to the method of Sanger. An internal Sac I restriction site (GAGCTC) could be found starting with nucleotide 1202. No start signal (ATG) was present at the 5' end of the cDNA indicating that the cDNA clone is incomplete.

Sequence No 3. Nucleotide sequence of ara-3.

This insert contained 1501 bp. A comparison with the GenBank at NIH showed that the sequence was homologous at the 5' end with cDNA clones derived from the human gall-bladder and leg muscle, and at the 3' end with a second cDNAs clone derived from leg muscle. No biological function has yet been found for these proteins.

Sequence No 4. Nucleotide sequence of ara-4.

This clone has two internal SacI and one EcoRI restriction sites. The phage insert contains 1700 bp. A comparison with known DNA sequences showed that the sequence was homologous at the 5' end with a cDNA clone from a human brain (infant with muscle atrophy) and at the 3' end with different cDNA clones derived from human tissues (brain of a healthy infant (Khan et al., 1992), keratinocytes (one clone) and unknown tissue (two clones). The biological function of the corresponding proteins have so far not been determined.

Sequence No 5. Nucleotide sequence of ara-5.

This clone was 900 bp and contained an internal SacI restriction site. 500 bp were sequenced. The sequenced part was homologous to human keratin type II.

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Sequence No 1. cDNA and d duced amino acid sequence of IgE-autoantigen Ka fused to β-galactosidase

```
... GGT GGC GAC GAC TCC TGG AGC CCG TCA GTA TCG GCG GAA TTC
    gly gly asp asp ser trp ser pro ser val ser ala glu phe

CGG TCC AAA CTG GGT CTT CGG CAG GTT ACA GGA GTT ACT AGA GTC   45
arg ser lys leu gly leu arg gln val thr gly val thr arg val

ACT ATC CGG AAA TCT AAG AAT ATC CTC TTT GTC ATC ACA AAA CCA   90
thr ile arg lys ser lys asn ile leu phe val ile thr lys pro

GAT GTC TAC AAG AGC CCT GCT TCA GAT ACT TAC ATA GTT TTT GGG  135
asp val tyr lys ser pro ala ser asp thr tyr ile val phe gly

GAA GCC AAG ATC GAA GAT TTA TCC CAG CAA GCA CAA CTA GCA GCT  180
glu ala lys ile glu asp leu ser gln gln ala gln leu ala ala

GCT GAG AAA TTC AAA GTT CAA GGT GAA GCT GTC TCA AAC ATT CAA  225
ala glu lys phe lys val gln gly glu ala val ser asn ile gln

GAA AAC ACA CAG ACT CCA ACT GTA CAA GAG GAA GTG AAA TTG GCG  270
glu asn thr gln thr pro thr val gln glu glu val lys leu ala

AGG AAA ACC TGA AAA TAG GTG GAA AAT TTA GAA ATG TCC ACT GTA  315
arg lys thr  *

GGA CGT GGA ATA TGG CAA GAA AAA CAT CGA ATT C
```

Sequence No 2. cDNA and deduced amino acid sequence of IgE-autoantigen Kb fused to β-galactosidase

```
... TCC TGG AGC CCG TCA GTA TCG GCG GAA TTC CGC GGA ATT CCG
    ser trp ser pro ser val ser ala glu phe arg gly ile pro

GGG CGG GAG CGC AGC CAG GCA GAG CCC TCC GAG CGG CGC GTG AAG   45
gly arg glu arg ser gln ala glu pro ser glu arg arg val lys

CGG GAG AAG CGC GAT GAC GGC TAC GAG GCC GCT GCC AGC TCC AAA   90
arg glu lys arg asp asp gly tyr glu ala ala ala ser ser lys

ACT AGC TCA GGC GAT GCC TCC TCA CTC AGC ATC GAG GAG ACT AAC  135
thr ser ser gly asp ala ser ser leu ser ile glu glu thr asn

AAA CTC CGG GCA AAG TTG GGG CTG AAA CCC TTG GAG GTT AAT GCC  180
lys leu arg ala lys leu gly leu lys pro leu glu val asn ala

ATC AAG AAG GAG GCG GGC ACC AAG GAG GAG CCC GTG ACA GCT GAT  225
ile lys lys glu ala gly thr lys glu glu pro val thr ala asp

GTC ATC AAC CCT ATG GCC TTG CCG ACA CGA GAG GAG CTG CGG GAG  270
val ile asn pro met ala leu pro thr arg glu glu leu arg glu

AAG CTG GCG GCT GCC AAG GAG AAG CGC CTG CTG AAC CAA AAG CTG  315
lys leu ala ala ala lys glu lys arg leu leu asn gln lys leu

GGG AAG ATA AAG ACC CTA GGA GAG GAT GAC CCC TGG CTG GAC GAC  360
gly lys ile lys thr leu gly glu asp asp pro trp leu asp asp

ACT GCA GCC TGG ATC GAG AGG AGC CGG CAG CTG CAG AAG GAG AAG  405
thr ala ala trp ile glu arg ser arg gln leu gln lys glu lys

GAC CTG GCA GAG AAG AGG GCC AAG TTA CTG GAG GAG ATG GAC CAA  450
asp leu ala glu lys arg ala lys leu leu glu glu met asp gln

GAG TTT GGT GTC AGC ACT CTG GTG GAG GAG GAG TTC GGG CAG ATG  495
glu phe gly val ser thr leu val glu glu glu phe gly gln met

GCG TGC AGG ACC TGT ACA GTG CCC GGG ACC TGC AGG GCC TCA CTG  540
ala cys arg thr cys thr val pro gly thr cys arg ala ser leu
```

Sequence No 2 (cont). cDNA and deduced amino acid sequ nc of IgE-autoantigen Kb fused to β-galactosidase

TGG AGC ATG CCA TTG ATT CCT TCG AGA AGG GAG ACA ATG ATT CTT 585
trp ser met pro leu ile pro ser arg arg glu thr met ile leu

ACC CTC AAG GAC AAA GGC GTG CTG CAG GAG GAG GAG GAC GTG CTG 630
thr leu lys asp lys gly val leu gln glu glu glu asp val leu

GTG AAC GTG AAC CTG GTG GAT AAG GAG CGG GCA GAG AAA AAT GTG 675
val asn val asn leu val asp lys glu arg ala glu lys asn val

GAG CTC CGG AAG AAG AAG CCT GAC TAC CTG CCC TAT GCC GAG GAC 720
glu leu arg lys lys lys pro asp tyr leu pro tyr ala glu asp

GAG AGC GTG GAC GAC CTG GCG CAG CAA AAA CCT CGC CTC TAT CCT 765
glu ser val asp asp leu ala gln gln lys pro arg leu tyr pro

GTC CAG TAT GAC GAA GAG CTT GAA GGG GAG CGG CCA CAT TCC TTC 810
val gln tyr glu glu leu glu gly glu glu arg pro his ser phe

CGC TTG GAG CAG GGC GGC ACG GCT GAT GGC CTG CGG GAG CGG GAG 855
arg leu glu gln gly gly thr ala asp gly leu arg glu arg glu

CTG GAG GAG ATC CGG GCC AAG CTG CGG CTG CAG GCT CAG TCC CTG 900
leu glu leu ile arg ala lys leu arg leu gln ala gln ser leu

AGC ACA GTG GGG CCC CGG CTG GCC TCC GAA TAC CTC ACG CCT GAG 945
ser thr val gly pro arg leu ala ser glu tyr leu thr pro glu

GAG ATG GTG ACC TTT AAA AAG ACC AAG CGG AGG GTG AAG AAA ATC 990
glu met val thr phe lys lys thr lys arg arg val lys lys ile

CGC AAG AAG GAG AAG GAG GTA GTA GTG CGG GCA GAT GAC TTG CTG 1035
arg lys lys glu lys glu val val val arg ala asp asp leu leu

CCT CTC GGG GAC CAG ACT CAG GAT GGG GAC TTT GGT TCC AGA CTG 1080
pro leu gly asp gln thr gln asp gly asp phe gly ser arg leu

CGG GGA CGG GGT CGC CGC CGA GTG TCC GAA GTG GAG GAG GAG AAG 1125
arg gly arg gly arg arg arg val ser glu val glu glu glu lys

GAG CCA GTG CCT CAG CCC CTG CCG TCG GAC GAC ACC CGA GTG GAG 1170
glu pro val pro gln pro leu pro ser asp asp thr arg val glu

Sequence No 2 (cont). cDNA and deduced amino acid sequence of IgE-autoantigen Kb fused to β-galactosidas

AAC ATG GAC ATC AGT GAT GAG GAG GAA GGT GGA GCT CCA CCG CCG 1215
asn met asp ile ser asp glu glu glu gly gly ala pro pro pro

GGG TCC CCG CAG TGC TGG AGG AGG ACG AGG CGG AGC TGG AGC TGC 1260
gly ser pro gln cys trp arg arg thr arg arg ser trp ser cys

AGA AGC AGC TGG AGA AGG GAC GCC GGC TGC GAC AGT TAC AGC AGC 1305
arg ser ser trp arg arg asp ala gly cys asp ser tyr ser ser

TAC AGC AGC TGC GAG ACA GTG CCA AGA AGG TGG TGG AGA TTG TGA 1350
tyr ser ser cys glu thr val pro arg arg trp trp arg leu *

AGA AGC TGG AGT CTC GCC AGC GGG GCT GGG AGG AGG ATG AGG ATC 1395

CCG AGC GGA AGG GGG CCA TCG TGT TCA ACG CCA CGT CCG AGT TCT 1440

GCC GCA CCT TGG GGG AGA TCC CCA CCT ACG GGC TGG CTG GCA ATC 1485

GCG AGG AGC AGG AGG CGG AAT TC

Sequenc No 3. cDNA of clon ara-3

GAA TTC CGG GCC ATC GAG AAA GTG CGG AAA TGG GAG AAG AAG TGG
GTG ACT GTG GGT GAC ACG TCC CTG AGG ATA TTT AAG TGG GTT CCT
GTG ACA GAC AGC AAG GAG AAA GAA AAG TCA AAA TCG AAC AGT TCA
GCA GCC CGA GAA CCT AAT GGC TTT CCT TCT GAT GCC TCA GCC AAT
TCC TCT CTC CTT CTT GAA TTC CAG GAC GAA AAC AGC AAC CAG AGT
TCC GTG TCT GAC GTC TAT CAG CTT AAG GTG GAC AGC AGC ACC AAC
TCA AGC CCC AGC CCC CAG CAG AGT GAG TCC CTG AAG CCC AGC ACA
CAC CTC CGA CTT CCG CAC GGA TGA CTC CCA GCC CCC AAT GGG CCA
GGA GAT CCT GGA GGA GCC CTC CCT GCC CTC CTC GGA AGT TGC TGA
TGA ACC TCC TAC CCT CAC CAA GGA AGA ACC AGT TCC ACT AGA GAC
ACA GGT CGT TGA GGA AGA GGA AGA CTC AGG TGC CCC GCC CCT GAA
GCG CTT CTG TCT GGA CCA ACC CAC AGT GCC GCA GAC GGG TCA GAA
AGC TAG CAC CAT CCC GGC CCT CCG CCT CCT GGC CCT GCC TCT ATT
TAT TGC ATT CTG GTT CTG GCC GCG CCG CGT TGC TGG GGA AGG GCA
AGC ACT GGG GTC AAG AGC CTG CAC ACA TGA GCC TTC CGG GCT GGA
AGG CTG GCG TAG GAC TTG GGG CTG TAG CAT CAT CTT CCT GAC CCT
GGC ACC TGT GTC TAC TTG CTC CCG AGA AGA GGA GCG CTC ATG TCT
TTT TTG CAC CCC AAG TTG GCT GGA GCA TCG GCC ACC CCA AGA TTC
ATC TGT GAC CTC CAG GCA GCA GTC TCT GCT CCA GAA TCT CTG GAC
GGA GCT GCT GGC AGC TTC TGC GAG AAG AGA GAG ATG TGG AAG GCA
CCT TCT AGA AGA GAG CGT GCC TCA GGT TAC TTG AAC TTG AAC GGA
GAC TGT AGA CTC CCG GAC TTT CCC CTA GGA CTG GGG GCC CTG TAG
GCT GCT GTT GGA GGA CTG GGT AGA GAC ATT GGA GGG AAG GGA AGG
GCT TTT CTC CAC ACA AGG GC AGA GAG TCC GTC TAG ATT TCT TGC
TGT CCT GCC AGC TCT GCC CAT GCC TGA GGT GGT CCT ACC TCT CAC
GGG CAC CCT AGC TGC TGA CAA CCC TTT GTG GCC GCC GTC CCC ATC
CCC TGC CCT CAG CAC ACA CAT CTG CAC ACA CGC GAC TTT GTT CTC

Sequence No 3 (cont). cDNA of clone ara-3

ACC TCT ACC TGT CAT TCC AGC ATC CCT GCC TCT TGT CAC AAA CTG
CCC CAG CAA GAA TTT GAG GTT CTG ACA ACA GTA CCC ATC CCC CAC
AGT ACC CCT TCA GCT CAG TTT CTA GAA AGC TCC CTT TTC TTT GAA
ATC TGC ATG TTG AAT TGA ACT TTG TGA TTT TAT TTT TTG TTT CAA
AAA AGT TTA AGA AAA TGG AAA TGG GCA ACA GTG AGT GAA GAC ATA
TTT TAG CAC TGA ATA GAA TAT TTT TAA AAT TAA ACT ATT TGA AAT
ATG AAA AAA CGG AAT TC

Sequence No 4. cDNA of clone ara-4

TTG ATG GAA AGA AAA TTT CCA GGA ACG AGA AAA ATT TGC TGA TGA
AGG CAG TAT ATT TTA CAC CCT TGG AGA ATG TGG GCT CAT ATC CTT
TTC AGA CTA CAT TTT CCT CAC AAC TGT TCT TTC CAC TCC TCA GAG
AAA TTT TGA AAT TGC CTT CAA GAT GTT TGA TTT GAA TGG AGA TGG
AGA AGT AGA TAT GGA AGA ATT TGA ACA GGT TCA GAG CAT CAT TCG
CCT CCC AAA CCA GTA TGG TAT GCG CCA CAG AGA TCG CCA ACT ACT
GGC AAC ACC CCT CAA GCC TTG CTT GTG TTC AGC CCT CAC AAC CTA
CTT TTT TGG AGC TGA TCT GAA GGG AAA CGT GAC AAT CAA AAC CTT
CCT CGA ATT TCA GCG TAA ACT GCA GCA TGA TGT CTG AAG CTT GAG
TTG ... ... GGC ACA CCT CAA GTC TGG CTG TGT CAG CCC TCC CAA
CCT ACT TTT GGA GCT GAT CTG AAG GGA AAG CTG ACC AAT CAA AAC
CTT CCC TCG AAT TTC ACC AAA CTG CAG CAT GAT GTT CTG AAG CTT
GAG TTG AAC GCC ATG ACC CTG TGG ATG GGA GAA TTA CTG AGA GGC
AGT TTG GTG GCA TGC TAC GTG CCT ACA GTG GGG TGC AGT CCA AGA
AGC TGA CCG CCA TGC AGA GGC AGC TCA AGA AGC ACT TCA AAG AAG
GAA AGG GTC TGA CAT TTC AGG AGG TGG AGA ACT TCT TTA CTT TCC
TAA AGA ACA TTA ATG ATG TGG ACA CTG CAT TGA GTT TTT ACC ATA
TGG CTG GAG CAT CTC TTG G ... ... *GAG CTC* TCA GAC CAC GTG
TGT GAT GTG GTG TTT GCA CTC TTT GAC TGT GAT GGC AAT GGC GAA
CTG AGC AAT AAG GAA TTT GTT TCC ATC ATG AAG CAA CGG CTG ATG
AGA GGC CTG GAA AAG CCC CAA AGA CAT GGG TTT CAC TCG CCT CAT
GCA GGC CAT GTG GAA AGG GCA CAG GAA ACT GCC TGG GAC TTG CGC
TTT ACC CAA ACA GTA ACC CCA CAC TGC AAG AAG GGG ACC CCT TCC
ACC CCA GTA CCC TGG ACC CCC TCC TGC AGA GTC TCG CAG AGC CCT
TTG TGC TGC TGC TTC TGG AAG TCG TCC CCC TTC TTC CCG GGA TGA
CCT CAG GAC TCT GTC GGT TTT CCC CTT CTT TAC CCT TCC CCT GGT
TCC CCG GTG GTC TGC TGG GCT CTG ATT CTT GCC CAA TTG AGG TTA

Sequenc No 4 (cont). cDNA of clone ara-4

TCC CCA TAG GTT CTT CAA AAA CAT GAA CAA GTC TGT AAA GCT TCA
GAC ATT TGT CAG CCT CAA CAG CAC CTT ACC CAT TCA AGC ATC CTG
TGG ATA AAG *AAT TC* ... ... TCC AAG GCC TGC TCT AGG AAG GCA
GCA TGC TCA GTG GGA ACA CAG CAA GAT TCA GAA TTT AAG TAG TTG
CTT CAT GGC TCT GTG CAC TCC CTT TTC TTC CTC GCA GCC TCC CTA
AGA TGA CT CCA GTG TGA CCC TGT GCT TAG TGT GCA ATA GTG ATT
GAG CTC ATG TTC CCT GCA ATG TCC ATT TAC TCT CCA GGA TGG GCC
TCT AAA GCT GAG GCC TGG CTC AGA GCC TGT TTG CCC TCT GTC TTA
AAC AAT TGT AAA TAT CAC TTA AAT TAT AAC CAT TTG CAA TAA ACA
TCC CCA AAG TTA AAA AAA AAA AAA AAA CGG *AAT TC*

Sequence No 5. cDNA of clone ara-5

GGC CGA GGA GCG TGC AAG GAT CAA GAC CCT CAA CAA CAA GTT TGC
CTC CTT CAT CGA CAA GGT GCG GTT CCT GGA GCA GCA GAA CAA GGT
TCT GGA AAC AAA GTG GAC CCT GCT GCG GAG CAG GGC ACC AAG ACT
GTG AGG CAG AAC CTG GAG CCG TTG TTC GAG CAG TAC ATC AAC AAC
CTC AGG AGG CAG CTG GAC AGC ATT GTC GGG GAA CGG GGC CGC CTG
GCA CTG AG ... ... CG CTC TCG GCT GCG GTA GTC AGG CAG TTG
AAT GAA GTG TTT ACC TTG TGG AGC GAC ATC CAG AGG CAC TTC ACT
TCG CAG CGG CTT ACC ATC CAG CGC CAC CTT CCA GTG CAG *GAG CTC*
... ... A AGG AGT ACC AGG AGG CTG ATG AAT GTC AAG CTG GCC
CTG GAC GTG GAG ATC GCC ACC TAC CGC AAG GCC TGC TGG AGG GTG
AGG GAG GGC AGG CTG AAT GGC GAA GGC GTT GGA CAA GTC AAC ATC
TCT GTG GTG CAG TCC ACC GTC TCC AGT GGC TAT GGC

PATENT CLAIMS

1. Recombinant DNA molecule comprising
   i) a nucleotide sequence which codes
      i:1) for a polypeptide displaying the antigenicity of the atopy related antigens Ka, Kb, ara-3 or ara-4 or
      1:2) for a polypeptide that is homologous to > 50 %, such as > 60 %, > 70 % or > 80 % to these atopy related antigens, or
      i:3) for a peptide comprising at least one epitope unique for (a) these atopy related antigens or for (b) a peptide that is homologous to > 50 %, such as > 60 %, > 70 % or > 80 % to the Ka or Kb antigen, or
   ii) a nucleotide sequence which hybridizes with such a sequence under conditions of high stringency.

2. Recombinant DNA molecule according to claim 1, comprising at last one of the nucleotide sequences set out in figures 1-4, respectively, or degenerative variants thereof, or one or more Ka, Kb, ara-3 or ara-4 unique epitopes of said sequences or variants.

3. Recombinant DNA expression vector or cloning system comprising an expression control sequence operatively linked to a nucleotide sequence coding a peptide and defined in (i) and (ii) of claim 1 or 2.

4. Host cell or host system containing a recombinant DNA molecule or a recombinant expression vector as defined in any of claims 1-3.

5. Polypeptide comprising at least one of the amino acid sequence defined by the nucleotide sequences coding for the atopy related antigens Ka, Kb, ara-3 and ara-4, or at least one epitope unique for these atopy related antigens.

6. Polypeptide according to claim 5 wherein the polypeptide has been recombinantly or synthetically produced.

7. Polypeptide according to any of claims 5-6 comprising at least one of the amino acid sequences encoded by the nucleotide sequences set out in figures 1-4, respectively, or an epitope thereof unique for the anyone of the atopy related antigens Ka, Kb, ara-3 or ara-4.

8. Polypeptide according to any of claims 5-7 wherein the peptide is derivatized in the sense that it is a) linked to a water-insoluble phase by physical adsorption or by covalent bonding, b) conjugated covalently to an analytically detectable group (label), or c) covalently linked to an additionally polypeptide.

9. Polypeptide according to claim 8 wherein it is recombinantly produced in the form of a fusion protein comprising the additional protein.

10. Polypeptide according to claim 9 wherein said additional polypeptide is β-galactosidase, GST or lambda cII protein or any other polypeptide which can be expressed in a prokaryotic or an eukaryotic cell.

11. Polypeptide according claim 8 wherein the water-insoluble phase is a water-insoluble polymer that may have a physical form selected from: walls of microtitre wells, spheres, rods, sheets, strips, pads etc, said physical forms may be porous or non-porous.

12. Polypeptide according claim 8 wherein the water-insoluble phase is a water-insoluble polymer selected from water-insoluble forms of polysaccharides and their derivatives, for instance dextran, pullulan, agarose, cellulose etc, or synthetic polymers, preferably vinyl polymers, such as

polyacrylamides, polyacrylates, polystyrene, polyvinyl alcohol etc.

13. Method for in vitro diagnosis of atopy or inflammation in an a mammalian individual, often a human individual, which method comprises detection of the reaction of IgE in a body fluid sample from the individual with an IgE autoantigen from the same species as the individual or an IgE hapten/autoantigen crossreacting with the IgE autoantigen, optionally derived from another source, wherein the formation of an IgE immune complex is taken as an indication that the individual is suffering from atopy, in particular atopic dermatitis or asthma bronchiale, or inflammation.

14. Method according to claim 11 wherein the autoantigen is according to any of claims 5-12 or ara-5 as definied in figure 5 including an IgE epitope of ara-5.

15. Method according to any of claims 13-14 wherein the body fluid sample is derived from blood, such as a whole blood sample, a serum sample or a plasma sample.

16. Method according to any of claims 13-15 wherein the autoantigen in insoluble form or in insolubilizable form is brought into contact with the sample under conditions permitting formation of an immune complex between the autoantigen and antibodies present in the sample, whereafter the amount of complexed IgE antibodies is detected, where appropriate after insolubilization of the formed IgE-autoantigen complex.

17. Method according to any of claims 13-15 wherein the autoantigen is soluble and labeled with an analytically detectable group, and the immune complex between the autoantigen and sample IgE is formed in a soluble form that in the course of being quantitated is insolubilized by being

contacted with water-insoluble or water-insolubilizable anti-IgE antibodies, preferably linked to the solid phases given in claim 12.

18. Method to measure in vitro the cellular reaction against an IgE-autoantigen, in which a polypeptide according to any of claims 5-12 is used to stimulate or to inhibit the cellular reaction.

19. Method of treatment of an mammalian individual, in particular a human being, suffering from atopy wherein an effective amount of a polypeptide according to any of claims 5-12 is administered to said individual.

20. Method of diagnosing atopy or inflammation, which comprises measuring an atopy related antigen as defined in claims 5-12, or an antibody, preferably of IgE class.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/00723

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/435, C07K 16/00, G01N 33/564, A61K 38/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, Volume 370, August 1994, Brigitte Wiedmann et al, "A protein complex required for signal-sequence-specific sorting and translocation" --	5
A	Science, Volume 253, August 1991, Rudolf Valenta et al, "Identification of Profilin as a Novel Pollen Allergen; IgE Autoreactivity in Sensitized Individuals" page 557 - page 560	1,5
X	see fig 2 --	13,15-17

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 November 1995	29-11-1995
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl Olof Gustafsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/00723

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, file 351, Derwent WPI, Dialog accession no. 009884024, WPI accession no. 94-163938/20, KURARAY CO LTD: "New peptide(s) and their salts binding to IgE - useful for diagnosis and treatment of allergic diseases", & JP, A, 6107685, 940419, 9420 (Basic)	13,15-18
A	National Library of Medicine, file Medline, accession no. 94365321, Saeki H et al: "HLA and atopic dermatitis with high serum IgE levels", & J Allergy Clin Immunol 1994 Sep;94(3 Pt 2):575-83	1,5
A	National Library of Medicine, file Medline, accession no. 94365320, Saeki H et al: "Polymorphisms of transporter associated with antigen processing genes in atopic dermatitis", & J Allergy Clin Immunol 1994 Sep;94(3 Pt 2):565-74	1,5
A	WO 9321223 A1 (BOARD OF REGENTS OF THE UNIVERSITY OF OKLAHOMA), 28 October 1993 (28.10.93), see pages 1-6 and claims	1,13
A	WO 8907601 A1 (TAN, KIM, SZE), 24 August 1989 (24.08.89), see page 7 and page 11, lines 31-34	13,15-17
A	Dermatology, Volume 189, No 1, 1994, T. Kawashima et al, "Impact of Ultraviolet Radiation on the Cellular Expression of Ro/SS-A-Autoantigenic Polypeptides" page 6 - page 10	1,13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/00723

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 19
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1 (iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [X] Claims Nos.: 1-3
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see extra sheet.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet.

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment f additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/00723

Box I

The wording "a nucleotide sequence which codes for ... a polypeptide that is homologous to > 50 % such as > 60 % ... "does not restrict the homology to those parts of the molecule that displays the relevant epitopes, making the scoop of the claim too vague to be adequately searched. This vague definition is further broadened by the expression "comprising at least one epitope unique for ... (b) a peptide that is homologous to ..." as this definition could well exclude any original epitope referred to above (i.e. non-atopy related epitopes are covered by the definition as "unique for ... a peptide"), not to mention the last definition of "high" stringency hybridizing nucleotide sequences covered by alternative ii). The search has been restricted to the sequences given in the description.

Box II

The present application refers to recombinant DNA molecules coding for several separate polypeptides displaying antigenicity of atopy related antigens Ka, Kb, ara-3 or ara-4, corresponding polypeptides and a method for in vitro diagnosis of atopy or inflammation comprising detection of the reaction of IgE in a sample with these autoantigens or with a further autoantigen, ara-5.

As it has been shown that some atopic diseases are associated with IgE autoreactivity against human profilin (see pages 1-2 of the description), the four different antigens referred to in claims 1-12 are considered to represent four independent inventions. No unifying concept except for the already known autoreactivity of IgE with epitopes of human origin. Accordingly, the present application refers to five different inventions, namely:

1. Ka antigen, corresponding recombinant DNA, expression vector and host cell according to claims 1-12 and a method for in vitro diagnosis with the aid of this antigen according to claims 13 and 15-20.
2. Kb antigen, corresponding recombinant DNA, expression vector and host cell according to claims 1-12 and a method for in vitro diagnosis with the aid of this antigen according to claims 13 and 15-20.
3. Ara-3 antigen, corresponding recombinant DNA, expression vector and host cell according to claims 1-12 and a method for in vitro diagnosis with the aid of this antigen according to claims 13 and 15-20.
4. Ara-4 antigen, corresponding recombinant DNA, expression vector and host cell according to claims 1-12 and a method for in vitro diagnosis with the aid of this antigen according to claims 13 and 15-20.
5. A method for in vitro diagnosis of atopy or inflammation comprising detection of the reaction of IgE in a sample with the ara-5 antigen according to claims 14-20.

A further search covering inventions was deemed to be possible to within one extra fee.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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